Eco. Env. & Cons. 30 (May Suppl. Issue) : 2024; pp. (S42-S46)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i03s.009

Impact of bio-priming (*Rhizobium leguminosarum*) to improve seedling vigour and germination potential to overcome abiotic stress in green gram

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(Received 9 August, 2023; Accepted 13 October, 2023)

ABSTRACT

Diverse seed priming methods are used to ameliorate seed germination, seedling vigour, and to overcome abiotic stress. Insertion to these, only the bio-priming system provides the fresh benefit of biotic stress operation, earning it special attention. Bio-priming is applicable in nearly all crops around the world and is an environmentally friendly volition to chemical pesticides. Seed bio-priming generally refers to use of salutary microorganisms to influence germination potential. In this study, *Rhizobium leguminosarum* were used in different concentration following Complete Randomized Design with three replications. Green gram (TMB-37) was collected from AICRP pulses in BCKV, Mohanpur, Nadia, West Bengal for this investigation. The experiment comprised of seven bio-priming treatments along with control. The seeds were subjected to *in-vitro* studies and data were analysed statistically. Seed bio-primed with *Rhizobium leguminosarum* @ 20 percent recorded higher seed germination percentage (82.71 %), shoot length (25.49 cm), seedling fresh weight (2.77 g), seedling dry weight (0.24 g), seedling vigour index I (3467.91) and seedling vigour index II (19.85). The results of this study demonstrated that *Rhizobium leguminosarum* @ 20 percent was the best.

Key words: *Bio-priming, Germination, Green gram, Rhizobium leguminosarum, Seed quality*

Introduction

Green gram [*Vigna radiata* (L.) Wilczek] is commonly known as mungbean, golden gram, mung or moong (John, 1991). With a total production of 16.43 lakh tonnes and an average productivity of 488 kg ha^{-1}, it is the third most significant pulse crop farmed in India on an area of 33.70 lakh hectares (Anonymous, 2017). In Indian states like Orissa, Andhra Pradesh, Maharashtra, Karnataka, and Bihar, it is widely grown. With a total production of 1.15 lakh tonnes and an average productivity of 277 kg ha^{-1} in Karnataka, it covers an area of 4.15 lakh hectares. Productivity declines are caused by a lack of high-quality seeds, the lack of appropriate seed production technology, heavy flower dropping, high insect and disease incidence, insufficient post-harvest handling procedures, and farmers' lack of variety knowledge. A sturdy seedling produced by high-quality seed is essential for India's agricultural success since it increases yield (Chakraborty and Bordolui, 2021). According to Sathiya *et al*. (2017), the use of subpar seed, poor crop management, and cultivation in parched, marginal soils were the main causes of pulses' low productivity. By combining conventional and scientific methods to achieve the crop's commercial success, it might be improved by developing appropriate low cost seed production technology. Seed priming is a controlled hydration procedure that involves exposing seeds to low water potentials that limit germination but allow pre-germinative physiological and biochemical changes (Khan, 1992). One priming technique that improves the characteristics of seeds and seedlings is bio priming. It's a method of biological seed treatment that involves combining seeds and injecting them with beneficial organisms to protect them against microbial attack. It is an ecological strategy that uses particular fungi as disease antagonists in the soil and in seeds ((Taylor and Harman, 1990).

Chemical control could be substituted with biological seed treatments utilising *Rhizobium leguminosarum*. To determine the impact of bio priming on the quality of green gram seeds, an in vitro experiment was conducted in 2022. Taking into consideration the previously mentioned details, the current experiment was conducted in a lab setting following seed priming with *Rhizobium leguminosarum* in a different dose with dry seeds serving as a control.

Materials and Methods

The laboratory experiment was carried out in seed testing laboratory, Department of Seed Science and Technology, BCKV, Mohanpur, Nadia, West Bengal, India during 2022 following Complete Randomized Design with three replications. Green gram (TMB-37) was collected from AICRP pulses, BCKV for this investigation. Seed priming was done with the solution of different concentration of *Rhizobium leguminosarum.* Dry seed was considered as control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); *Rhizobium leguminosarum* 10% (T_3); *Rhizobium leguminosarum* 15% (T_4); *Rhizobium leguminosarum* 20% (T_5); *Rhizobium leguminosarum* 25% (T_6); *Rhizobium leguminosarum* 30% (T_7). For each treatment, seeds were soaked for 8 hours. The seeds were then removed from the solution, tested for germination on standard germination papers using the Petri plate and glass-plate methods, and incubated for 7 days at 25 degrees Celsius with a relative humidity of 90% in a seed germinator. Seed germination percentage (%), shoot length (cm), root length (cm), seedling length (cm), seedling dry weight (g), seedling vigour index I (Abdul-Baki and Anderson, 1973), and seedling vigour index II observations on seed quality parameters were recorded.

Germination Parameters

Seedling parameters

Root lengths and shoot lengths of ten seedlings were measured at 7 days after germination by glass plate method in the laboratory with the help of a scale and graph paper and average was made out, expressed in centimetre (cm). Fresh weight of ten seedlings was measured with the help of a digital balance. Then seedlings were dried at 60-70 °C for two hours in hot air oven and weighed in a digital balance. Both seedling fresh weight and dry weight are expressed in gram (g).

Germination percentage

Germination percentage (G) was calculated as:

$$G = \frac{\text{Number of normal seedlings produced}}{\text{Total number of seeds used}} \times 100$$

Where, X is the number of normal seedlings produced and Y denotes total number of seeds taken for germination (ISTA, 1996). It is expressed in percentage.

Vigour Index-I

Vigour index (VI) was calculated by using the formula suggested by Abdul Baki and

Anderson (1973): VI- I = G X L

Where, 'G' indicates germination percentage and 'L' denotes average seedling length (cm).

Vigour Index-II

It was also calculated by Abdul Baki and Anderson (1973) using the formula:

VI-II = G x DW (10 seedlings).

Where, 'G' indicates germination percentage and DW denotes dry weight.

Results

Germination percentage

Significant responses were noticed in the priming treatment of *Rhizobium leguminosarum* solution with different concentration of under laboratory condition. T_5 (82.710%) recorded highest germination percentage followed by T_4 and T_2. While lowest germination percentage was recorded for T_1 (77.33) preceded by T_3 and T_7 respectively. But, non-significant difference was observed in between T_1 and T_3; T_2, T_3,

T_4, T_6, T_7 and T_4 and T_5 (Table1). Similar kind of experiment on chickpea was observed by Choudhury and Bordolui (2022b).

Root length (cm)

Significant difference was observed in root length for this bio priming. Maximum seedling root length was observed for T_4 (18.08 cm) followed by T_5 and T_6 respectively, while it was minimum for T_1 (10.287 cm) (Table 1). Although T_2 and T_3 showed non-significant difference was observed among themselves. The results are in agreement with the fact that root and shoot length increased in seeds due to priming as compared to non-primed seeds reported by Novak *et al*. (2009); Choudhury and Bordolui (2022a).

Shoot length (cm)

In case of shoot length, the longest seedling shoot length was recorded for T_5 (25.49 cm) followed by T_4 and T_6 while shortest shoot length was observed in T_7 (19.48 cm) preceded by T_1 and T_2 (Table 1). Significant difference was noted for shoot length in overall though non-significant difference was observed in between T_4 and T_6. The result corroborates the findings of Srimathi *et al*. (2007).

Fresh weight (g)

Significant difference was observed in fresh weight after *Rhizobium leguminosarum* priming. Highest seedling fresh weight was observed for T_5 (2.774 g) followed by T_4 and T_3 while lowest was noted in T_1 (1.32 g) proceeded by T_2 and T_7 respectively. But non-significant difference was noticed in between

Table 2. Effect of bio-priming on fresh weight, dry weight, and vigour index-II of Green gram

Treatment	Fresh weight (g)	Dry weight (g)	Vigour Index-II
T_1	1.32	0.16	12.36
T_2	1.50	0.186	14.906
T_3	2.146	0.194	15.174
T_4	2.654	0.214	17.34
T_5	2.774	0.24	19.846
T_6	2.046	0.206	16.42
T_7	1.694	0.14	11.10
SEm (±)	0.18	0.018	1.422
LSD (0.05)	0.550	0.054	4.356

Note: Control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); *Rhizobium leguminosarum* 10% (T_3); *Rhizobium leguminosarum* 15% (T_4); *Rhizobium leguminosarum* 20% (T_5); *Rhizobium leguminosarum* 25% (T_6); *Rhizobium leguminosarum* 30% (T_7).

T_1, T_2 and T_7; T_3 and T_4; T_3 and T_6 and T_4 and T_5 (Table 2). The result was in agreement with Bordolui *et al*. (2018).

Dry weight (g)

In case of dry weight, it was significantly varied due to priming with different concentration of *Rhizobium leguminosarum*. Maximum seedling dry weight was noticed for T_5 (0.24 g) followed by T_4 and T_6 respectively while minimum was noticed for T_7 (0.14 g) preceded by T_1 and T_2 respectively. But, non-significant difference was observed in between T_1, T_2, T_3, T_4 and T_6; T_1, T_2, T_3 and T_7 (Table 2). Similar kind of experiment reported Mohammadi (2009) in soybean (*Glycine max* L.); Ray and Bordolui (2022) in tomato.

Table 1. Effect of bio-priming on germination percentage, root length, shoot length and vigour index-I of Green gram

Treatment	Germination Percentage (Tr value)	Shoot length (cm)	Root length (cm)	Vigour Index-I
T_1	77.33 (61.546)	21.573	10.287	2,463.83
T_2	79.683 (63.198)	22.197	14.203	2,899.86
T_3	78.397 (62.285)	23.657	14.56	2,996.38
T_4	81.37 (64.412)	24.33	18.08	3,450.87
T_5	82.71 (65.41)	25.49	16.44	3,467.91
T_6	79.573 (63.114)	24.313	15.417	3,161.75
T_7	79.213 (62.856)	19.487	13.123	2,582.76
SEm (±)	0.548	0.198	0.255	35.805
LSD (0.05)	1.678	0.607	0.782	109.654

Note: Control (T_1); Seeds soaked in distilled water/ hydropriming (T_2); *Rhizobium leguminosarum* 10% (T_3); *Rhizobium leguminosarum* 15% (T_4); *Rhizobium leguminosarum* 20% (T_5); *Rhizobium leguminosarum* 25% (T_6); *Rhizobium leguminosarum* 30% (T_7).

Vigour Index-I

Among the priming treatments, with different duration and concentration of *Rhizobium leguminosarum* significant difference was observed in Vigour Index-I. Maximum value was calculated for T_5 (3,467.91) followed by T_4 and T_6 respectively. while it was minimum for T_1 (2463.83) preceded by T_7 and T_2 (Table 1). Although, vigour index was significantly varied, but some non–significant difference was also noticed in between T_2 and T_3; T_4 and T_5. This result corroborates the findings of Kamaraj and Padmavathi (2012) in green gram.

Vigour Index-II

Vigour index-II significantly varied due to priming with different concentration of *Rhizobium leguminosarum*. Lowest Vigour index-II was observed in T_7 (11.10) proceeded by T_1 and T_2. While, T_5 (19.846) showed the highest germination index followed by T_4 and T_6. But, non-significant difference was observed in between T_1, T_2, T_3 and T_6; T_1, T_2, T_3 and T_7; T_2, T_3, T_4 and T_6; T_3, T_4, T_5 and T_6 (Table 2). Similar type of result was noticed by Sujaya *et al*. (2018).

Discussion

Rhizobium leguminosarum have the ability to create definite phytohormones such as auxin, gibberellin, ethylene, cytokinin, abscisic acid. It has also ability to alter the production of phytohormones secreted by plants and thus play several roles in plant growth and development (Vacheron *et al*. 2013). *Rhizobium leguminosarum* have a capacity of stimulation in cell division, distinctions in meristematic tissues on the root, root hair augmentation, decrease on inhibiting lateral root formation, reducing root-shoot ratio and induce shoot growth (Vacheron *et al*. 2013).

Rhizobium leguminosarum can adjust to help in regulating water uptake ensuring that seeds imbibe water at an optimal rate. Controlled hydration through bio-priming triggers various physiological processes required for germination, such as the mobilization of reserves, respiration, and cell expansion. It strengthens the uniformity and speed of germination, resulting in more vigorous seedlings (ISTA, 1996).

Conclusion

The increased levels of hormones such gibberellins that were activated in the seeds treated with *Rhizobium leguminosarum* @ 20% may have triggered the action of specific enzymes that promoted early germination, such as amylase, resulting in the improved seed quality features. As a result, assimilations of starch would have been readily available. The completion of pre-germination metabolic activities during seed priming gets the seed ready for germination and resulting in the highest germination rate. Therefore, pre-sowing treatment with 20% *Rhizobium leguminosarum* for 8 hrs is recommended for better performance of Green gram as it is superior to other treatments including control, in terms of enhancing seed germination and seedling vigour.

Acknowledgement

Authors are thankful to Department of Seed Science and Technology, BCKV, Mohanpur, Nadia, West Bengal.

Conflict of Interest: The author(s) certify that they have No Conflict of Interest.

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